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Chemical signals from conspecfics modify the activity of female bank voles *Clethrionomys glareolus*

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The influence of chemical cues from conspecifics on female bank voles *Clethrionomys glareolus* (Schreber, 1780) activity was investigated in a 10 min behavioural test. The role of the main olfactory and vomeronasal systems in mediating chemicals which alter female activity was also studied. Total activity scored higher in females exposed to the scent of dominant male or adult male urine. The odour of subordinate male, castrated male and female urine had no effect on female activity. Bulbectomy but not vomeronasalectomy decreased female activity in the presence of an adult male. The results are discussed in terms of possible biological functions of such behaviour.

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Introduction

There is a considerable body of information on bank vole *Clethrionomys glareolus* (Schreber, 1780) reproductive activity, social organization and behaviour in natural populations or outdoor enclosures (reviewed in Viitala and Ylönen 1993, Ylönen and Mappes 1995). It has been demonstrated that at least males of *Clethrionomys* species form stable dominance hierarchies in the field (Viitala 1977 for *C. rufocanus*) and in the laboratory (Gustaffson *et al.* 1980 for *C. glareolus*).

Several findings suggest that chemical signals may be at least partly involved in the regulation of reproductive physiology and behaviour in bank voles (Hoffmeyer 1982, Kruczek and Marchlewska-Koj 1986, Kruczek *et al.* 1989). It has been shown that adult male urine and secretions of the salivary and preputial glands contain chemical signals able to attract females (Kruczek 1994a).

The temporal pattern of activity is one of the basic characteristics of any animal species. The activity pattern observed on a particular day is not fixed. It is a product of interaction between the endogenous rhythm and the modifying effect of external factors (Hall 1995a, b). However, there is little information in the literature concerning the effect of chemical signals on animal activity.

The vomeronasal system has been shown to be especially important for the perception of chemicals involved in reproductive processes (Wysocki 1979, Meredith 1991, Wysocki and Lepri 1991), whereas the main olfactory system appears to be responsible for food detection (Leung *et al.* 1972) and individual recognition (Gheusi *et al.* 1994). However, the function of these two systems sometimes overlaps (reviewed in Pfeiffer and Johnston 1994).

Bank vole females reared singly show very low levels of locomotor activity (M. Kruczek, unpubl.). The purpose of this study was to determinate if the odour of conspecifics modifies female activity and to identify which olfactory system, main or vomeronasal, alters female activity.

Material and methods

Animals

The bank voles used in these experiments were from an outbred stock colony reared in the Department of Mammalian Reproduction, Institute of Zoology, Jagiellonian University. The animals were maintained in metal cages (40 × 20 × 25 cm) at 18–20°C on a photoperiod schedule of 14 h light : 10 h dark. All bank voles were provided *ad libitum* water and food (Rabbit chow, Motycz, Poland), supplemented with a piece of apple once a week. The wood shavings used as bedding material were changed once a week. The females and males were kept 2 or 3 per cage; they were reared in groups of the same sex from weaning at 21 days. At least two weeks prior to each experiment, each 2- to 3-month-old female was individually housed.

All females and males used in present experiments, except the males tested for their social rank, were sexually non-experienced. A total of 76 intact, 9 sham-operated (SHAM), 9 vomeronasal-ectomized (VNX), and 6 olfactory bulbectomized (OBX) females were used (Permission no 29/95 of the Jagiellonian University Rector's Permanent Committee on the Bioethics of Animal Experimentation).

Thirty-seven males were used as intact males and 87 were castrated. Twenty males were used to establish their social rank. Five intact adult males and 5 adult females were used as urine donors. For urine collection the animals were kept for 24 h in metabolic cages.

Surgical procedure and histological verification

All operated animals were anaesthetized with pentobarbitone sodium (0.1 ml/10 g body weight, Polfa, Poland). The males were castrated at 8 weeks of age and then left undisturbed for 3 weeks. The vomeronasal organ was removed in a standard procedure described for prairie voles *Microtus ochrogaster* by Wysocki *et al.* (1991). Briefly, the females were placed supine in a stereotaxic apparatus, the mouth was opened and a midline incision was made through the soft tissues of the palate to expose the incisive bone, while a constant vacuum cleared any blood that appeared near the incision. A dental drill was used to remove the exposed bone covering the vomeronasal capsule to allow access to the vomeronasal organ, which was then extracted. The wound was closed with cyanoacrylate gel.

The bulbectomy was performed according to the technique of Leung *et al.* (1972). The females were placed in a stereotaxic apparatus, a cranial midline incision was made, and after the overlying cranial vault was removed the olfactory bulbs were removed by aspiration. The residual cavity was filled with gel foam. The anaesthesia and incision for the SHAM procedure were identical to the OBX procedure but the olfactory bulbs were left intact. All females were allowed at least 2 weeks to recover from surgery.

Surgeries were histologically verified upon termination of the experiments by a method described earlier (Kapusta *et al.* 1996). Only complete VNX and OBX females were used for further analysis. Two females had incomplete VNX and behavioural data collected from these animals were excluded from consideration.

Tests

Rank test

To determine the social hierachy the males were reared with females for at least two weeks before the ranking test. Then the social rank was established by the method described by Huck (1982) for mice. Briefly, the same two males were paired in a neutral arena for 10 min on each of 10 consecutive days. During each encounter, aggressive behaviour expressed in the number of attacks was noted for both males. An animal was classified as dominant if it showed more attacks than the other member of the pair.

Behavioural test

For total activity measurement intact, Sham-operated (SHAM), vomeronasalectomized (VNX) and bulbectomized (OBX) females in the presence of different stimulus males were transferred from their home cages into glass vivaria (33 × 19 × 27 cm). The floor of each vivarium was covered with clean wood shavings which were changed for each test. Following 4 h of habituation, a pair of stimulus males anaesthetized with pentobarbitone sodium (Polfa, Poland) were introduced to the vivarium.

The pairs of stimulus males were: (1) dominant and castrated, (2) subordinate and castrated, (3) two castrated, (4) castrated and castrated covered with intact male urine, (5) castrated and castrated covered with adult female urine. The samples of 0.5 ml urine were applied onto the anogenital region of the castrated males.

The female behaviour was taped with a PANASONIC M5 video camera for 10 min beginning from her first approach with both males. The number of approaches and sniffing behaviour of females towards the two stimulus males was noted from a TV monitor. Approach was defined as the directional locomotion towards stimulus male whereas sniffing behaviour was recorded when the female nose contacted the test male within ca 0.5 cm of his body. Total activity was taken as the sum of approaches and sniffing behaviour exhibited during the test.

Behavioural data were analysed by one-way analysis of variance (ANOVA) and post-hoc comparisons were made with Duncan's multiple range test. Comparisons between dominant and subordinate males were made using the *t*-test (Sokal and Rohlf 1981). Statistical significance was established at $p < 0.05$.

Results

The pairs of stimulus males displayed stable dominant-subordinate relationships during the ranking test. The number of attacks presented by one male in the pair of males, referred to later as dominant, were higher than those of the subordinate on all days of the ranking test. Only on the first day this difference was not significant (Fig. 1).

As shown in Table 1, bank vole females tested in the presence of an anaesthetized dominant male or male urine showed significantly higher total activity than females exposed to an anaesthetized subordinate male, two castrated males, or a castrated male and covered with female urine.

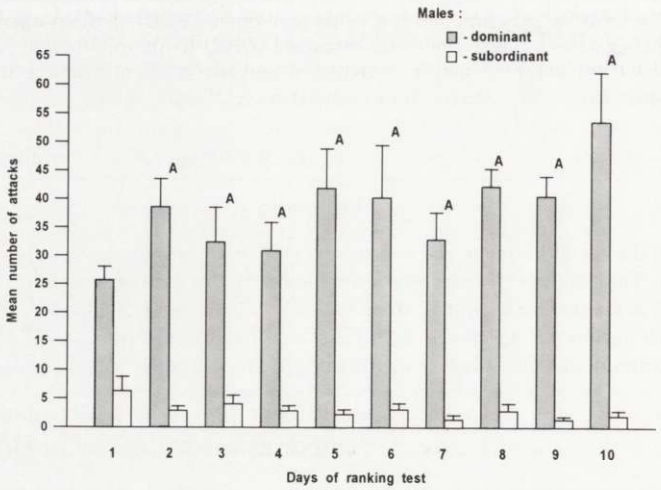

Fig. 1. The number of attacks (mean ± SE) of dominant and subordinate bank vole males during 10 min encounters in 10 consecutive days of ranking tests. A – $p < 0.01$ (t–test). Number of tested pairs of males: $n = 10$.

Total activity was a function of the total number of sniffing behaviours. Females exposed to a dominant male or male urine made significantly more sniffs than those tested in the presence of a subordinate male or castrated male, as well as in the presence of female urine.

Lesions of the olfactory systems by bulbectomy influenced the female's total activity in the presence of adult males (Table 2). OBX females scored lower total

Table 1. The total activity and sniffing behaviour (mean ± SE) of bank vole females tested in presence of two anaesthetized stimulus males. Those means marked by the same symbols differ significantly from each other: a, b – $p < 0.05$; A, B, C, D, E, F – $p < 0.01$.

Tested pairs of males	No. pairs	Total activity	Sniffing behaviour
dominant castrated	10	39.3 ± 5.0^{ABC}	24.1 ± 2.8^{ABC}
subordinate castrated	10	21.6 ± 3.5^{Ab}	12.5 ± 2.0^{AD}
castrated castrated	11	19.5 ± 2.6^{BD}	11.0 ± 1.5^{BE}
castrated + male urine castrated	12	34.1 ± 4.8^{Dab}	23.2 ± 3.5^{DEF}
castrated + female urine castrated	10	19.9 ± 2.4^{Ca}	11.5 ± 1.5^{CF}
		$F_{(4,48)} = 5.54$ $p < 0.01$	$F_{(4,48)} = 7.16$ $p < 0.01$

Table 2. The total activity and sniffing behaviour (mean ± SE) of sham-operated (SHAM), vomeronasalectomized (VNX) and bulbectomized (OBX) bank vole females in presence of two anaesthetized stimulus males – castrated and intact. Those means marked by the same symbols differ significantly from each other: A, B – $p < 0.01$.

Female condition	No. pairs	Total activity	Sniffing behaviour
SHAM	9	42.8 ± 3.0^{A}	21.8 ± 1.7^{A}
VNX	9	41.8 ± 3.5^{B}	27.4 ± 2.7^{B}
OBX	6	$19.5 \pm 2.9^{A,B}$	$6.2 \pm 1.2^{A,B}$
		$F_{(2,21)} = 13.89$	$F_{(2,21)} = 22.95$
		$p < 0.01$	$p < 0.01$

activity than SHAM or VNX females. Bulbectomized females also showed significantly fewer sniffing behaviours in comparison with VNX or SHAM females.

Discussion

Most laboratory tests have employed the reaction of a single animal to another single animal or single factor. Under natural conditions the social situation is probably much more complicated. Free-living female rodents often encounter a problem not present in a two-animal test: they must select the odours of different sexes and select the male with which to mate. That is why our behavioural test used more than one stimulus male. Since the synthesis and secretion of bank vole male sex-attractant is testosterone-dependent, castrated males were used in all tests as hormonally inactive males (Kruczek 1994a).

The results clearly show that the total activity of bank vole females is highly dependent upon chemical cues released by conspecifics. The higher total activity scores of females exposed to the scent of dominant male or intact male urine reflected higher numbers of sniffing behaviours. The scent of a subordinate male as well as the urine of adult females did not increase female activity above the level observed in the presence of castrated males. This is consistent with Hoffmeyer's (1982) finding that chemical signals from subordinate bank vole males are less attractive to females than are those from dominant males. Olfactory cues associated with social dominance also play an important role in sexual behaviour, as is well documented for the brown lemming *Lemmus trimucronatus* (Huck and Bank 1982).

In many rodent species the females may play an active role in mate selection (Getz *et al.* 1981). Dominant males may bear genes that produce fitter offspring and/or may be more successful in gaining and defending resources. Thus, the offsprings of females that discriminate, that are attracted to and mate with dominants, will have an advantage over those that do not. The higher total activity of bank vole females in the presence of a dominant male may be regarded as the first mate-selection gestures.

The vomeronasal system was not essential in mediating the male chemosignals that stimulate female bank vole behaviour. This finding accords with the observation of Lepri *et al.* (1990) that total locomotor activity of VNX prairie vole *Microtus ochrogaster* females exposed to males was not changed in comparison with SHAM-operated females. However, vomeronasalectomy eliminated the ability of bank vole females to recognize the hormonal state of the male (Kruczek 1994b). Only complete olfactory bulbectomy decreased female activity in the presence of an adult male. On the other hand, olfactory bulbectomy has been found to elevate the long-term activity of single-reared male mice (Possidente *et al.* 1996).

In the field, female bank voles are territorial (Bujalska 1985, Ylönen and Viitala 1985), and during the breeding season their home ranges overlap with the males' territory (Pusenius and Ylönen 1994). In *Clethrionomys* species, eg *Clethrionomys rufocanus* and *Clethrionomys glareolus*, territorial females seem to form the basis of the population and pregnancy seems to be possible only for females holding territories (Viitala and Hoffmeyer 1985). Bank vole males signal their territories by urinary marking, including deposition of preputial gland secretions (Christiansen 1980, Rozenfeld *et al.* 1986, Rozenfeld and Rasmont 1991). But the mechanism involved in female spacing behaviour is still not well known. Rozenfeld and Denoël (1994) found that breeding female bank voles mark their territories with urine but only in response to the odour of familiar female.

The present results may suggest higher female bank vole activity elicited by chemosignals from hormonally active males as a possible element in the maintenance of territorial boundaries, however, further experimentation is needed to test this possibility.

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